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The synthesis of heat-shock proteins by Aiptasia pulchella and its algal endosymbiont, symbiodinium sp. in response to thermal stress and the relationship of the heat-shock response to the bleaching of reef corals

5. FUNDING NUMBERS
N00014-92-J-1856-P00001

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13. ABSTRACT (Maximum 200 words)

1. We have characterized genes encoding heat shock proteins and proteins of the ubiquitin system. Constitutive forms of the HSP60 and 70 families of heat shock proteins and an inducible form of HSP 60 occur in symbiotic and aposymbiotic *Aiptasia pallida.*
2. We have constructed cDNA libraries from aposymbiotic *A. pallida* and symbiotic *A. pallida* and *Anthopleura eligantissima.* We have a complete sequence for *A. pallida* HSP60
3. We have completed the comparison of protein profiles of *A. eligantissima* (see J. Exp Biol. 1996, 199:883-892) and have identified three symbiosis-specific proteins. One is carbonic anhydrase with an apparent molecular weight of 31 kDa and a pI of 6.3. We have obtained N-terminal sequence for second protein; it has an apparent molecular weight of 32 kDa and a pI of 7,9. It does not align with any sequences in the GenBank data base. A third symbiosis-specific protein, having an apparent weight of 30 kD and a pI of 5.6, crossreacts with a monoclonal anti-HSP70 antibody. The synthesis of this protein is enhanced in animals subjected to elevated temperatures and in animals maintained in the dark. Further investigations of these proteins are underway by Dr. Virginia Weis at Oregon State University.

14. SUBJECT TERMS	15. NUMBER OF PAGES
Heat shock proteins, symbiotic and apo-symbiotic cnidarians, *Aiptasia pallida,* *Anthopleura eligantissima,* synbiosis-specific proteins, cDNA libraries,	5
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Final Report for the Office of Naval Research

Contract #: N00014-92-J-1856

Principal Investigator: Dr. Paul Levine
Institution: Hopkins Marine Station, Stanford University
Email: plevine@leland.stanford.edu,
Grant Title: The regulation of gene expression in cnidarian-algal associations

Award Period: 1 July 1995 - 30 June 1998

Objectives: A. To identify and characterize heat shock protein genes that are induced during elevated temperature in *Aiptasia pallida*, B. Compare and contrast the protein profiles of symbiotic and aposymbiotic *Anthopleura elegantissima*; and C. Identify and characterize the symbiosis-specific proteins, their functions and the genes that encode them.

Accomplishments
We have made a great deal of progress on all three of our objectives this year and they are outlined below by objective.

A. Heat shock:

We have been characterizing the genes that encode the heat shock proteins and those of the ubiquitin system, and we are determining changes in their expression during thermal stress. Members of the HSP60 and 70 families of heat shock proteins were identified by immunoblots. Constitutive forms of both were present in both symbiotic and aposymbiotic *A. pallida* as well as an inducible form of HSP60 that was induced by thermal stress. We have constructed a high titer cDNA library, containing 8 X 10^7 recombinant clones, from aposymbiotic *A. pallida* RNA using Stratagene's λZAP cDNA synthesis system. The library was screened for sequences that encode ubiquitin, UBCs, and HSPs. Positive clones for HSP60 and UBC have been subcloned into pBluescript for sequencing and we have a complete sequence for HSP60. We have also constructed cDNA libraries from symbiotic *A. pallida* and symbiotic *A. elegantissima.*

B. Comparison of protein and transcript profiles between symbiotic and aposymbiotic animals:

We completed the comparison of protein profiles and published the study in *J. Exp. Biol.*

We had a polyclonal antiserum made against symbiotic host homogenate that we then complexed with aposymbiotic homogenates. The conjugates were precipitated out of solution and the resulting supernatant was an antiserum enriched for symbiotic proteins. We have demonstrated, using immunoblots, that this enriched antiserum labels only symbiotic proteins. We are now ready to immunoscreen a cDNA library made from symbiotic host RNA to try to identify symbiosis-specific genes.

We have begun to compare transcript populations between symbiotic and aposymbiotic animals using a new technique called serial analysis of gene expression (SAGE). This PCR-based technique will allow us to compare transcript populations both quantitatively and qualitatively potentially yielding a wealth of information relating to both differential expression and the identity of transcripts. To date we have completed pilot experiments in which we have determined the amount of starting material necessary and the design for the linkers and primers.

C. Identification and characterization of symbiosis-specific proteins and their encoding genes.

We have identified one of the symbiosis-specific proteins from our comparative study as carbonic anhydrase (CA). The spot from symbiotic profiles at 31 kD and 6.3 pI crossreacts on immunoblots with a polyclonal rabbit anti-carbonic anhydrase. Further, a 16-peptide fragment from this spot (DFPAAAGARQSPIDIK)has a 70 - 80% identity with various vertebrate carbonic anhydrases. We have also localized this protein to the endodermal cells housing the symbiotic algae using the polyclonal antiserum in immunocytochemistry. In an attempt to obtain the cDNA for CA, we tried unsuccessfully to immunoscreen the cDNA library from symbiotic animal RNA with the CA antibody. However we will try another strategy in the coming year (see under work plan).

We have obtained N-terminal sequence from the 32 kD, 7.9 pI symbiosis-specific protein. It appears to be a novel protein as the 20 amino acid sequence (HGNLVEAKSLGLTDLISAXK) does not align with anything in the GenBank database. We have designed a degenerate primer to the 5' end that we will use with oligo dT to amplify a cDNA from RNA using the PCR, and then either clone and sequence the product or use it as a probe to screen the symbiotic cDNA library.

We identified a symbiosis-specific protein at 30 kD, pI 5.6 that crossreacts with a monoclonal anti-HSP 70 antibody. This protein is completely absent from aposymbiotic animals. Further, synthesis of this protein in symbiotic animals is enhanced in animals subjected to an elevated temperature stress and in animals that are kept in the dark.

Publications:

Weis, V. M., von Kampen, J., and R. P. Levine. In press. Techniques for Exploring Symbiosis-Specific Gene Expression in Cnidarian/Algal Associations. In: *Molecular Approaches to the Study of the Ocean.*. Ed. K. Cooksey, Chapman Hall, London.

Weis, V. M. and R. P. Levine. 1996 Differential Protein Profiles Reflect the Different Lifestyles of Symbiotic and Aposymbiotic *Anthopleura elegantissima*, a sea anemone from temperate waters. *J. Exp Biol.* 199(4):883-892.

Weis, V. M., S. Sanders, and D. Krupp. 1996 Larval development in the coral *Fungia scutaria* is affected by infection with symbiotic algae. *Proceedings Eighth International Coral Reef Symposium* . Abstract.

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To: plevine@leland.Stanford.EDU
From: Virginia Weis <weisv@ava.bcc.orst.edu>
Subject: report

Paul,

I am enclosing a final report as a word document attachment. Let me know right away if you can't read it. The name of the file is Levine final report.doc. You may need to change the award period information- currently it says 1 July 1995 - 30 June, 1998 - that closing date must be wrong but I don't know what it was. Also the last page - the silly graphics page, I'm sending that to you in the mail - can't send it as an attached file. It should arrive by Monday. Hope this is what they want and that it'll keep them happy.

Cheers,

Virginia

Levine final report.doc

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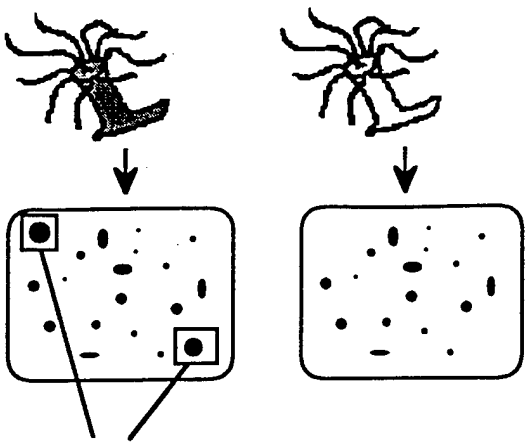

1. Perform 2D gel electrophoresis on symbiotic and aposymbiotic animals.
2. Identify unique proteins.
3. Purify and sequence unique proteins to gain insight into their function in the association.

Objectives

- Identify heat shock protein genes in symbiotic cnidarians.
- Identify and characterize symbiosis-specific genes and gene products in symbiotic cnidarians.

Accomplishments

- Cloned and sequenced HSP60 from a cDNA library of a symbiotic anemone.
- Found significant and repeatable differences in 2D protein profiles from symbiotic vs aposymbiotic anemones.
- Purified a symbiosis-specific protein from anemone homogenates.
- Collected aposymbiotic coral larvae and successfully infected them with symbiotic algae.

Significance

- Determine role of heat shock proteins in breakdown of cnidarian/algal symbioses.
- Identification of symbiosis-specific genes lends insight into inter-partner regulation and communication.

ATTACHMENT NUMBER 2

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